EXHIBIT A

(a)

(b)

(c)

(d)

Figure 17.14 Habitats of hyperthermophilic Archaea. (a) A typical solfatara in Yellowstone National Park. Steam rich in hydrogen sulfide rises to the surface of the earth. Because of the heat and acidity, higher forms of life do not develop. (b) Sulfur-rich hot spring, a habitat containing dense populations of *Sulfolobus*. (c) A typical boiling spring of neutral pH in Yellowstone Park, Imperial Geyser. (d) An iron-rich geothermal spring, another *Sulfolobus* habitat.

ferrous iron (aerobically) by various *Sulfolobus* species and the oxidation of H_2 or Fe^{2+} coupled to NO_3^- reduction (producing NO_2^- and eventually N_2 or NH_4^+) (see Table 17.6). Thus, a variety of respiratory processes can be carried out by hyperthermophilic Archaea, but in many cases elemental sulfur plays a key role, as either an electron donor or an electron acceptor.

Hyperthermophiles from volcanic habitats

As mentioned previously, volcanic habitats can have temperatures as high as 100°C and are thus suitable for hyperthermophilic Archaea. The first such organism discovered, *Sulfolobus*, grows in sulfur-rich hot acid springs (Figure 17.14*b*) at temperatures up to 90°C and at pH values of 1–5*. *Sulfolobus* (Figure 17.15*a*) is an obligate aerobe capable of oxidizing H_2S or S^0 to H_2SO_4 and fixing CO_2 as carbon source. *Sulfolobus* can also grow chemoorganotrophically. Cells of *Sulfolobus* are generally spherical but form distinct lobes (Figure 17.15*a*). Cells adhere tightly to sulfur crystals where they can be visualized microscopically by use of fluorescent dyes (∞ Figure 13.20*b*). Besides an active *aerobic* metabolism, *Sulfolobus* can also reduce Fe^{3+} to Fe^{2+} (but not grow) *anaerobically*. The ability of *Sulfolobus* to oxidize Fe^{2+} to Fe^{3+} aerobically (Figure 17.14*c*), however, has been used quite successfully in the high temperature leaching of iron and copper ores (∞ Section 14.17).

A facultative aerobe resembling *Sulfolobus* is also present in acidic solfataric springs. This organism, named *Acidianus* (Figure 17.15*b*), differs from *Sulfolobus* primarily by virtue of its ability to grow *anaerobically*

*Historical note: *Sulfolobus* was first discovered by Thomas Brock and colleagues in 1970 and formally described in 1972. The discovery of *Sulfolobus*, along with the previously isolated *Thermus aquaticus* (source of the extremely thermostable *Taq* DNA polymerase, see back cover of this book), is generally credited with launching the field of hyperthermophilic microbiology. Thomas Brock was the senior author of the first seven editions of this book. In the 1980s to the present, Karl Stetter and colleagues in Germany have greatly expanded the field of hyperthermophilic microbiology with the discovery of many new genera and species.

TABLE 17.6 Energy-yielding reactions of hyperthermophilic Archaea

Nutritional class	Energy-yielding reaction	Example
Chemoorganotrophic	Organic compound + $S^0 \rightarrow H_2S + CO_2$	*Thermoproteus, Thermococcus, Desulfurococcus, Thermofilum, Pyrococcus*
	Organic compound + $SO_4^{2-} \rightarrow H_2S + CO_2$	*Archaeoglobus*
	Organic compound + $O_2 \rightarrow H_2O + CO_2$	*Sulfolobus*
	Organic compound $\rightarrow CO_2$ + fatty acids	*Staphylothermus*
	Organic compound $\rightarrow CO_2 + H_2$	*Pyrococcus*
Chemolithotrophic	$H_2 + S^0 \rightarrow H_2S$	*Acidianus, Pyrodictium, Thermoproteus*
	$H_2 + NO_3^- \rightarrow NO_2^- + H_2O$ (NO_2^- reduced to N_2 by some species)	*Pyrobaculum, Stygiolobus, Aquifex, Pyrodictium, Thermoproteus*
	$4\,H_2 + NO_3^- + 2\,H^+ \rightarrow NH_4^+ + 3\,H_2O$	*Pyrolobus*
	$2\,H_2 + O_2 \rightarrow 2\,H_2O$	*Acidianus, Sulfolobus, Pyrobaculum, Aquifex*[a]
	$2\,S^0 + 3\,O_2 + 2\,H_2O \rightarrow 2\,H_2SO_4$	*Sulfolobus, Acidianus*
	$4\,FeS_2 + 15\,O_2 + 2\,H_2O \rightarrow 2\,Fe_2(SO_4)_3 + 2\,H_2SO_4$	*Sulfolobus*
	$10\,FeCO_3 + 2\,NO_3^- + 24\,H_2O \rightarrow 10\,Fe(OH)_3 + N_2 + 10\,HCO_3^- + 8\,H^+$	*Ferroglobus*
	$4\,H_2 + SO_4^{2-} + 2\,H^+ \rightarrow 4\,H_2O + H_2S$	*Archaeoglobus*
	$4\,H_2 + CO_2 \rightarrow CH_4 + 2\,H_2O$	*Methanopyrus, Methanococcus*

[a]Member of the Bacteria.

Remarkably, *Acidianus* is able to use S^0 in both its aerobic and anoxic metabolism. Under *aerobic* conditions the organism uses S^0 as an electron *donor*, oxidizing S^0 to H_2SO_4. Anaerobically, *Acidianus* uses S^0 as an electron *acceptor* (with H_2 as electron *donor*) forming H_2S as the reduced product. Thus, the metabolic fate of S^0 in cultures of *Acidianus* depends on the presence of O_2 and/or an electron donor.

Like *Sulfolobus*, *Acidianus* is roughly spherical in shape (Figure 17.15*b*). It grows at temperatures from about 65°C up to a maximum of 95°C, with an optimum of about 90°C. Another property shared by *Sulfolobus* and *Acidianus* is an unusually low GC base ratio. The DNA of *Sulfolobus* is about 38% GC, whereas that of *Acidianus* is even lower, about 31%; many other hyperthermophiles have DNA of low GC content as well (see Table 17.7). These low GC base ratios are intriguing when one considers the hyperthermophilic nature of these organisms; how do they prevent their DNA from melting? In the test tube, DNA of 30–40% GC content would melt almost instantly at 90°C. Obviously hyperthermophiles have evolved protective mechanisms to prevent DNA melting *in vivo* and we discuss these in Section 17.5.

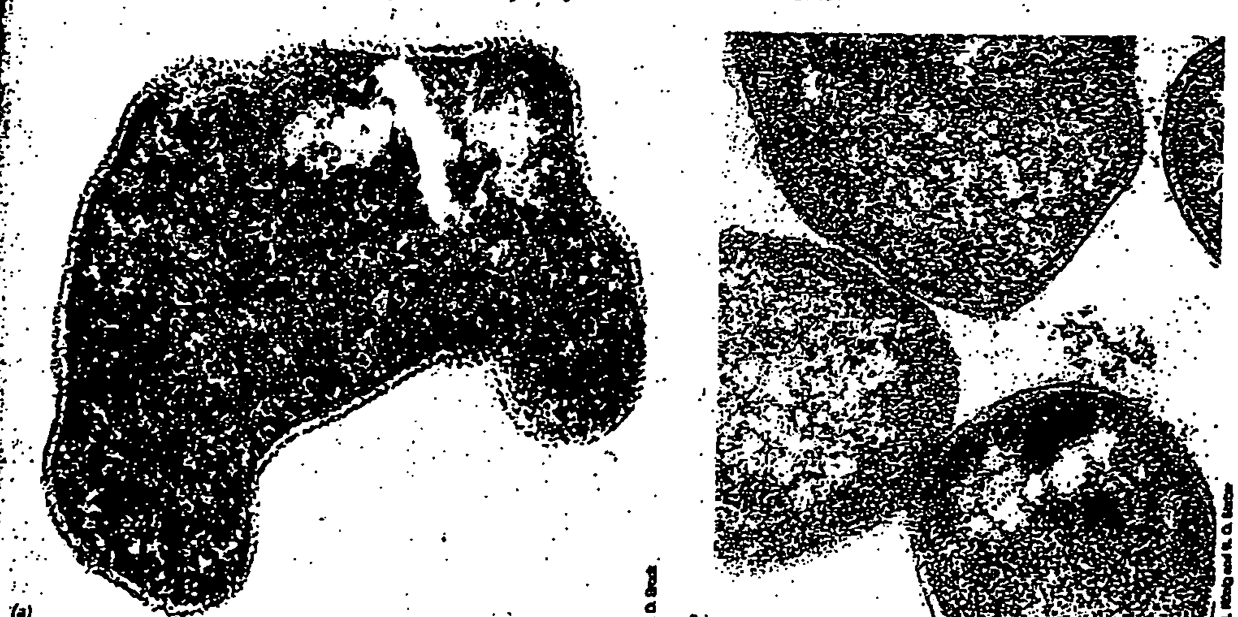

Figure 17.15 Acidophilic hyperthermophilic Archaea. (a) *Sulfolobus acidocaldarius*. Electron micrograph of a thin section. (b) *Acidianus infernus*. Electron micrograph of a thin section. Cells of both organisms vary from 0.8 to 2 μm in diameter.